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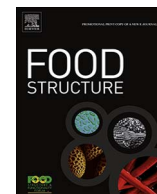
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Effect of alcohols on gellan gum gel structure: Bridging the molecular level and the three-dimensional network

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ABSTRACT

This work focuses on the understanding of the interaction of alcohols with gel systems during solvent exchange, following the gel formation. A method of widening the possible alcohol contents in formulations is proposed, as most hydrocolloids have a low tolerance of high alcohol concentrations and in some gelation is completely prevented.

Once the CPKelco LA (low-acyl or deacylated) gellan gum gel was produced, different alcoholic solvents (ethanol, 1-propanol, 2-propanol) were used to remove water from the material and replace it, investigating the effect on the gel network as a function of the alcohol chain length.

It is the first time that a research paper considers the high-alcohol/gellan gum systems at both the molecular and macroscopic scales, proposing the link between them. Specifically, the interaction of the alcoholic solvents with both the polymer chains and three-dimensional network was evaluated by characterising the physical and mechanical gel properties throughout the alcohol treatment.

From this study, the solvent effect on the gelling agent is evident, leading to structure shrinkage and distortion due to a high-induced stress on the gellan gum network. Gradual addition of the alcoholic solution was found to considerably reduce this behaviour due to the slower solvent exchange.

As evidence, and to further validate this study on LA gellan gum, both k-carrageenan and gelatin alcogels were investigated, since their gelation mechanism and molecular configuration are respectively similar and different to LA gellan gum. It was found that k-carrageenan reproduced the LA gellan results, unlike gelatin.

1. Introduction

The use of alcohols in combination with gelling agents has been increasing in different industrial fields. In the food industry, hydrocolloids are often used with ethanol in both food and beverage products to provide functional properties (Chaudhary, Small, & Kasapis, 2013; Imeson, 2012), such as the system homogeneity and stability over time (Chalupa, King, & Giampetro, 1997; Imeson, 2012), the viscosity enhancement (Khan, Park, & Kwon, 2007) and the soft tribology improvement (Mills, Koay, & Norton, 2013). Moreover, alcohols might be found in drying processes, such as in the supercritical-fluid drying of both gel systems (Scherer, 1990; Tkalec, Knez, & Novak, 2015; Ubeyitogullari & Ciftci, 2016) and entire food products (Brown, Fryer, Norton, Bakalis, & Bridson, 2008; Garcia-Gonzalez et al., 2007).

In the biomedical sector, alcohols may be used to dry and sterilise hydrogels (Perrut, 2012; Shearer, Ellis, Perera, & Chaudhuri, 2006) and to prepare them for microscopy characterisation (Allan-Wojtas, Hansen, & Paulson, 2008). Furthermore, in tissue engineering ethanol is

widely used in combination with biopolymers during scaffold preparation (Dai, Ronholm, Tian, Sethi, & Cao, 2016) and for the decellularisation process (Sawada, Terada, Yamaoka, Kitamura, & Fujisato, 2008).

Some hydrocolloids (e.g. pectin and guar gum) can still form a gel network if alcohols are added to the hot solution during the preparation stage (Oakenfull & Scott, 1984; Phillips & Williams, 2000; Tkalec et al., 2015). In this case, the used term is alcogel (Tkalec et al., 2015). However, the alcohol percentage depends on the specific gelling agent and can be limited. For instance, hydroxypropyl cellulose (HPC) is soluble in aqueous solution with an ethanol concentration around 50%, while xanthan gum water-solution can contain up to 60% in ethanol (Phillips & Williams, 2000). Furthermore, the ethanol addition can affect some gel properties, like transparency, and promote gelation at lower temperatures (Yamanaka et al., 2000).

In this context, a common gelling agent used in the food industry is LA (low-acyl or deacylated) gellan gum (Khan et al., 2007; Morris, Nishinari, & Rinaudo, 2012), which is a microbial polysaccharide,

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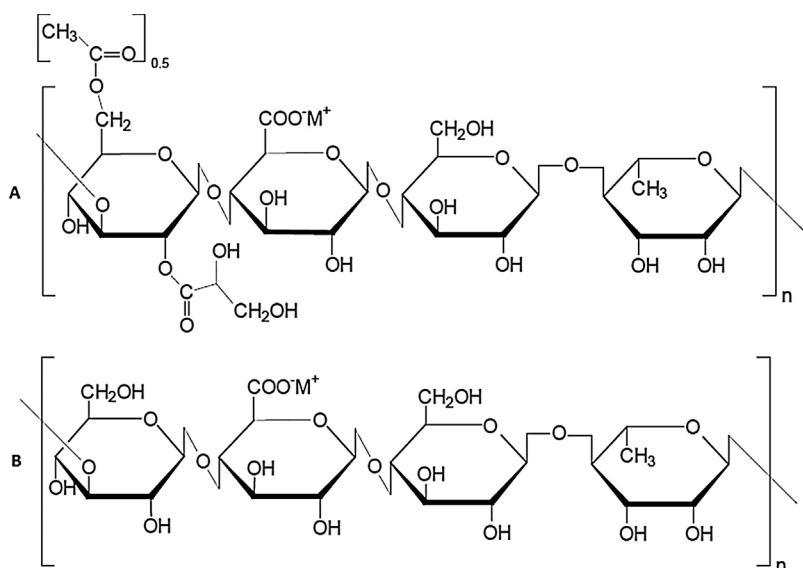


Fig. 1. HA gellan gum (A) and LA gellan gum (B) polymeric chains (adapted (Mahdi, Conway, & Smith, 2015)).

produced by the microorganism *Sphingomonas elodea* (previously identified as *Pseudomonas elodea*) in a fermentation process. The primary structure is a tetrasaccharide unit composed of glucuronic acid, rhamnose and glucose (Phillips & Williams, 2000), with a molecular weight range between 100 and 200 kDa (CPKelco, 2007; Kirchmayer, Steinhoff, Warren, Clark, & in het Panhuis, 2014). In the adapted Fig. 1 (Mahdi, Conway, & Smith, 2015), both the high-acyl (HA) and deacylated gellan gum chains are shown.

The two gellan gum types can be blended to provide synergistic properties to the system, especially in terms of mechanical properties (Phillips & Williams, 2000), whose ratio and total solid content depends on the specific application.

Morris, et al. (2012) reported that the gelation mechanism for gellan gum starts by formation of double helices, and, afterwards, the ion-induced association of the double helices leads to junction zone formation. In other words, the gellan network consists predominantly of flexible, disordered chains, with few ordered junction zones between the helices. For these zones, stabilising forces such as hydrogen bonds, electrostatic forces, hydrophobic interactions, Van der Waals attractions and molecular entanglement are defined by the solvent conditions and polymer structure (Hui, 2006). It is reported that low acyl gellan gum requires cations, acid, soluble solids or combinations of these additives (CPKelco, 2007; Morris et al., 2012; Norton, Cox, & Spyropoulos, 2011).

Since gellan gum is not soluble in ethanol (Bajaj, Survase, Saudagar, & Singhal, 2007), yet the product formulation may contain both the ingredients, this work proposes a method to widen the alcohol content in gelling agent systems. LA gellan gum was chosen as a gel model system, representing other hydrocolloids with similar gelation mechanism (Aguilera & Stanley, 1999). The characterisation at the molecular scale was performed by mDSC and FTIR, as Sudhamani, Prasad, and Udaya Sankar (2003) reported for the gellan gum gels without alcohols, whereas the mechanical properties were investigated by texture analysis.

The alcohol-hydrocolloid interaction was assessed when the gel was already produced. Particularly, quiescent gels (Morris et al., 2012) were evaluated to study the effect on the molecular/network structures. However, this study is also applicable to smaller aggregates, e.g. gel particle suspensions (Banerjee & Bhattacharya, 2012; Norton, Jarvis, & Foster, 1999).

2. Material and methods

2.1. Gel preparation and solvent treatment

Double distilled water, obtained by a water still system (Aquatron A400D, Stuart, UK), was heated up to 85 °C and then the low acyl gellan gum powder (Kelcogel F, CPKelco, UK) was slowly added to avoid clump formation. The polymer concentration was 2% w/w in order to have a stable quiescent gel block, yet not too dense. To ensure a complete hydration, the solution was stirred for two hours at constant temperature.

No salts were added to strengthen the gel (Phillips & Williams, 2000) in order to avoid introducing a further variable to the system, and potentially affecting the results on the solvent interaction with the gel network in both mechanical and chemical properties.

The solutions were poured into sample moulds (13.5 mm in diameter and 65 mm in height), which were covered with a plastic film to prevent evaporation. A cooling rate of around 0.5 °C/min down to room temperature (20 °C ± 1 °C) was recorded. This specific cooling rate was kept constant during all the experiments to minimise changes in the gel structure, as Nitta, Yoshimura, and Nishinari (2014) suggested.

After the gel setting, the moulds were stored at room temperature (20 °C ± 1 °C) for 24 h.

Afterwards, the obtained gel samples had dimensions of 13.5 mm in diameter and 10 mm in height.

Similarly, 2% w/w gelatin (from porcine skin, Sigma-Aldrich, UK) and 2% w/w k-carrageenan (Sigma-Aldrich, UK) were prepared to compare the solvent quality with LA gellan gum.

Once the gels were formed, different alcohols were separately used to assess their effect on the gel properties. Ethanol (EtOH), 1-Propanol (1-PrOH) and 2-Propanol/Isopropanol (2-PrOH) (AnalaR NORMAPUR, VWR, UK) were used as pure solvents or diluted in different concentrations with distilled water to perform a gradual alcohol treatment. In this specific case, solutions at 25, 50 and 80 wt% were prepared. When a gradual treatment was applied, the gel samples were left, stepwise, in the alcoholic solution for 6 h. Finally, the treated gels were submerged in the pure solvent. The last step of the gradual treatment was 24-h long. On the other hand, if the treatment was not gradual, the samples were directly left for 24 h in the specific alcoholic solution/pure alcohol.

2.2. Mechanical and shrinkage measurements

Mechanical properties were evaluated by analysing the material

texture. Particularly, both the Young's modulus and the gel strength produced by a strain compression of 50% were assessed.

The texture analyser was the TA.XT.plus (Stable Micro Systems Ltd., UK), and a 40 mm diameter cylindrical aluminium probe was fitted on it. In this way, the sample diameter was always kept at least twice smaller than the diameter of the probe. After the application of a thin layer of silicone oil on the probe plates (Bagley, Wolf, & Christianson, 1985), a compression rate of 2 mm/s (Norton et al., 2011) was set. All the measurements were carried out in triplicate for the statistical analysis.

The gel strength value is the force peak in the force-time graph, whereas the Young's modulus is measured by elaborating the “true strain” (ε_H)/“true stress” (σ_T) curve, using the following Eqs. (1)–(4) (Mao, Tang, & Swanson, 2000):

$$\varepsilon_E = (H_0 - h)/H_0 \quad (1)$$

$$\varepsilon_H = \ln(1 + \varepsilon_E) \quad (2)$$

$$\sigma_E = F/A_0 \quad (3)$$

$$\sigma_T = \sigma_E (1 + \varepsilon_E) \quad (4)$$

Where ε_E and ε_H are respectively the engineering and true strains, while σ_E and σ_T are the engineering and true stresses. A_0 and H_0 are the cross-sectional area and initial height of each sample. F and h are the recorded compression force applied and height of each sample.

To measure the shrinkage of gel, paraffin oil (Sigma-Aldrich) was used (Del Valle, Cuadros, & Aguilera, 1998), being hydrophobic and therefore it cannot easily penetrate the gel structure. The method used is based on the liquid displacement (Fig. 2) (Yan, Sousa-Gallagher, & Oliveira, 2008).

This method requires the measurement of M' (the mass of the chamber completely filled with oil), M_d (the mass of oil with the sample after treatment) and M_o (the oil mass with the non-treated sample).

The theoretical oil density lies in the range of 0.827 g ml⁻¹ and 0.890 g ml⁻¹, while the experimental one ρ_l was found to be 0.871 g ml⁻¹. Weight measurements were carried out before and after the solvent treatment and for each experiment, six measurements were collected for a statistical evaluation.

2.3. FTIR spectroscopy

Molecular interactions between the hydrocolloid and alcohols were evaluated by Fourier Transform Infrared Spectroscopy (Spectrum Two

IR Spectrometer, Perkin Elmer) in reflection configuration. Spectra were collected for gels, gels with solvents (alcogels), and alcohols with a resolution of 4 cm⁻¹.

The selected scanning range was 600–4000 cm⁻¹ wave numbers and 16 scans were applied to each sample.

2.4. Micro DSC

Thermal transitions at the molecular level were investigated by using a micro DSC 3 evo (Seteram Instrumentation). The gel samples were previously cut to fit the equipment cells. All the experiments with LA gellan gum and k-carrageenan were carried out from 5 °C to 80 °C with a scan rate of 1 °C/min and in each experiment two heating/cooling cycles were applied. Isothermal periods were performed to prevent the system from the thermal history effect. For gelatin, the maximum temperature was set at 60 °C.

3. Results and discussion

The solvent quality in LA Gellan gum was analysed on three different scales. Specifically, the chemical and physical properties of both the constitutive polymer molecule and gel network as well as the mechanics of the gel at a macroscopic level were considered.

3.1. Molecular level

In order to characterise the interaction of the solvent with the gellan gum chains and the effect on the gel network, infrared spectroscopy was performed (Brown et al., 2008). The peaks and intensity information is dependent on the macromolecule conformation and, therefore, the system is sensitive to the specific solvent (Pawde & Deshmukh, 2008).

Fig. 3 shows the collected spectra for pure ethanol, LA gellan gum hydrogel and alcogel. Results for LA gellan gum gel were in agreement with the current literature (Coutinho et al., 2010; Pikal & Shah, 1990; Sudhamani et al., 2003).

After the gel dehydration by ethanol treatment, a decrease in OH peak (between 3400 and 3200 cm⁻¹) was noticed (Fig. 3a), mainly due to the water removal and replacement with the alcohol.

At the same time, in the alcogel the typical ethanol bands in the fingerprint region were evident, between 1100 cm⁻¹ and 1000 cm⁻¹. In the alcogel, the characteristic ethanol peaks for the C–O stretch were shifted from 1095 cm⁻¹ and 1048 cm⁻¹ to 1075 cm⁻¹ and 1037 cm⁻¹ respectively, suggesting that the interaction between ethanol and the polymer chains occurred. Similar considerations were reported for the gellan gum-LiCF₃SO₃ system (Noor et al., 2012). Therefore, since ethanol is polar with a strong hydrogen-bond donor group (–OH) (Antoniu, Buitrago, Tsianou, & Alexandridis, 2010; Roberts & Caserio, 1977), it seemed that it effectively interacts with the polymer at the molecular level, leaving an imprint on the gel structure. The EtOH presence affected the typical gellan gum peak at 1637 cm⁻¹ related to the glycosidic bond (Noor et al., 2012; Sudhamani et al., 2003). In effect, the alcogel showed a peak shift to 1605 cm⁻¹, suggesting that the solvent was effectively interacting.

In addition, the EtOH alcogel peak at 1037 cm⁻¹ is slightly more intense than pure ethanol. This may indicate that more contributions were present, namely the C–O–C stretches along the polymer chain and C–O stretch in the ethanol molecule (Fig. 3b).

A deeper investigation of the ethanol/gellan gum interaction was carried out by analysing the micro DSC thermographs (Fig. 4a–b). Specifically, the effect of ethanol on the gel structure could be assessed by the evaluation of the thermal transitions that were involved during the gel cooling and heating.

It is well-known that the gelation of gellan gum leads to the aggregation of the double helices, forming the junction zones (Chandrasekaran, Millane, Arnott, & Atkins, 1988; Morris et al., 2012) during cooling. It was confirmed that gellan gum gelation occurred in a

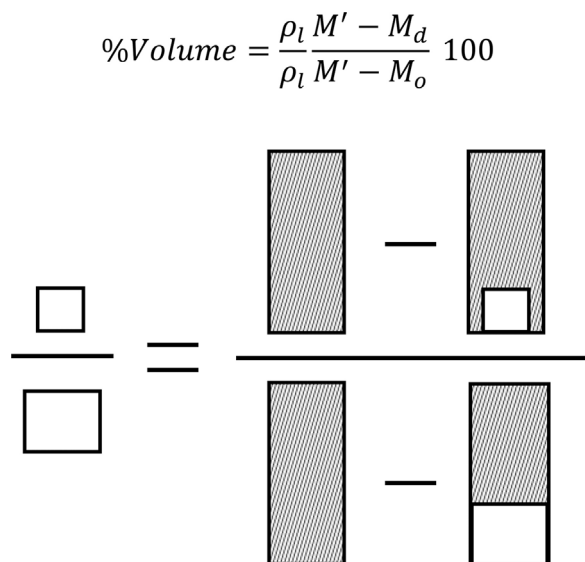


Fig. 2. Shrinkage measurement method: formula on the top.

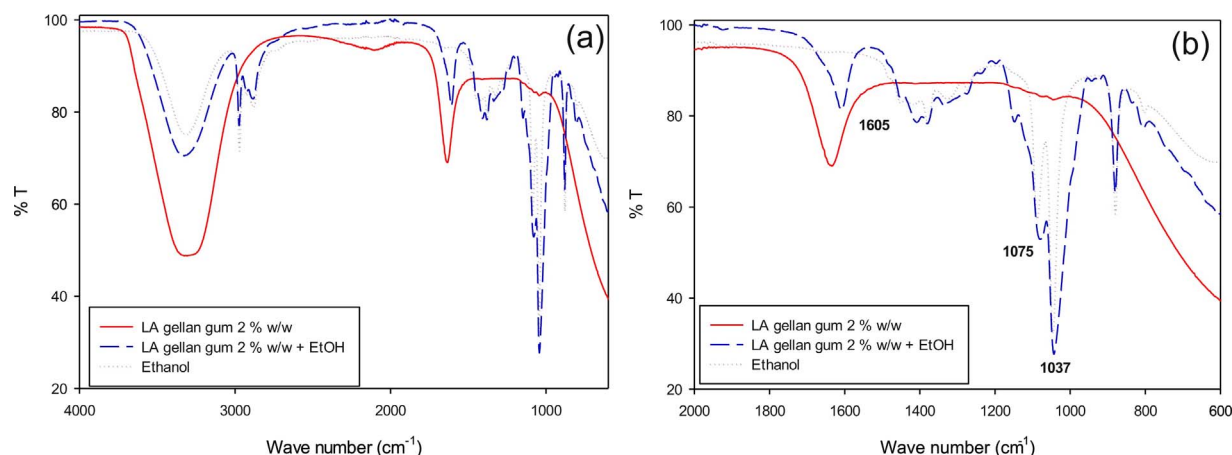


Fig. 3. a-b FT-IR spectra for LA Gellan gum and ethanol: left-(4000–600 cm^{-1}), right-(2000–600 cm^{-1}). The peaks values are related to the alcogel.

single exothermic transition and, therefore, the sol-gel transition took place at a temperature similar to the coil-helix transition one (Picone & Cunha, 2011). In effect, the shape of the peak might indicate that two transitions at slightly different temperatures occurred (Fig. 4b). On the other hand, on heating (Fig. 4a), the main endothermic peak at $\sim 30^\circ\text{C}$ is related to the disruption of helices mainly in a non-aggregated state (Moritaka, Nishinari, Taki, & Fukuba, 1995; Picone & Cunha, 2011), while the thermal transitions at higher temperatures are related to the aggregated double helices. Therefore, the presence of other peaks during the heating stage may suggest that the system is characterised by different thermal transitions at the molecular level (Moritaka et al., 1995; Picone & Cunha, 2011).

Since the thermal events were nearly flat when ethanol was used, it is clear that the molecular thermal transitions were affected. Particularly, both the endothermic and exothermic peaks are not present and it seems they are distributed over a wide temperature range. This consideration can be correlated to the effect of high sugar content in gel systems, as Chaudhary, et al. (2013) reported. This experiment may suggest that the thermoreversible behaviour is lost when ethanol is added, replacing water as a solvent. From this result, it is likely that a decrease in gellan network order occurs, namely due to the effect of ethanol on the structure.

Water in the three dimensional structure is mainly present as free water (Ablett & Lillford, 1991; Mashimo, Shinyashiki, & Matsumura, 1996) and it plays an key role in the formation of crystalline regions (Hatakeyama & Hatakeyama, 1998). In a true-quiescent gel, in parallel to the water movement in the gel network, water molecules can move

between the junction zones (Ohtsuka & Watanabe, 1996), hydrating the double helices (Ohtsuka & Watanabe, 1996). If salts are added to the gel solution, metal ions tend to replace water molecules between the helices (Ohtsuka & Watanabe, 1996), affecting as a consequence the stability of the junction zones (Morris et al., 2012). The addition of alcohols tend to alter the water network (Nose & Hojo, 2006), likely affecting the stabilising interactions and therefore the gel structure stability (Antonioni et al., 2010; Hui, 2006), decreasing the network order. Specifically, the water molecules removal by alcohols tend to destabilise the hydrogen bonds especially in the hydrophilic portions (Eltoum, Fredenburgh, Myers, & Grizzle, 2001). Additionally, this effect may results in a different mobility of the polymer chains when a stress is applied in alcohols rather than in water. In other words, the chain movement is more likely to be hindered by changing the solvent due to the molecular and network configuration in alcohols compared to water.

In this light, Fig. 5a shows the mDSC curves during cooling as a function of ethanol concentration in the aqueous solution. At 25 wt% EtOH, a reduction in the endothermic peak and a shift to a lower temperature were noted. It is ascribed to the formation of a more disordered structure by comparison with the non-treated gel, since a lower entropy reduction was quantitatively noticed. In effect, by using the alcoholic solution at 25 wt% EtOH, the ΔS value changed, calculated on cooling as $\Delta H/T$ at the equilibrium, from $-6.4 \cdot 10^{-3} \pm 0.3 \cdot 10^{-3} \text{ J g}^{-1} \text{ }^\circ\text{C}^{-1}$, referred to the non-treated gel, to $-4.7 \cdot 10^{-3} \pm 0.7 \cdot 10^{-3} \text{ J g}^{-1} \text{ }^\circ\text{C}^{-1}$. This means that after the treatment the entropy reduction related to the main thermal transition was lower.

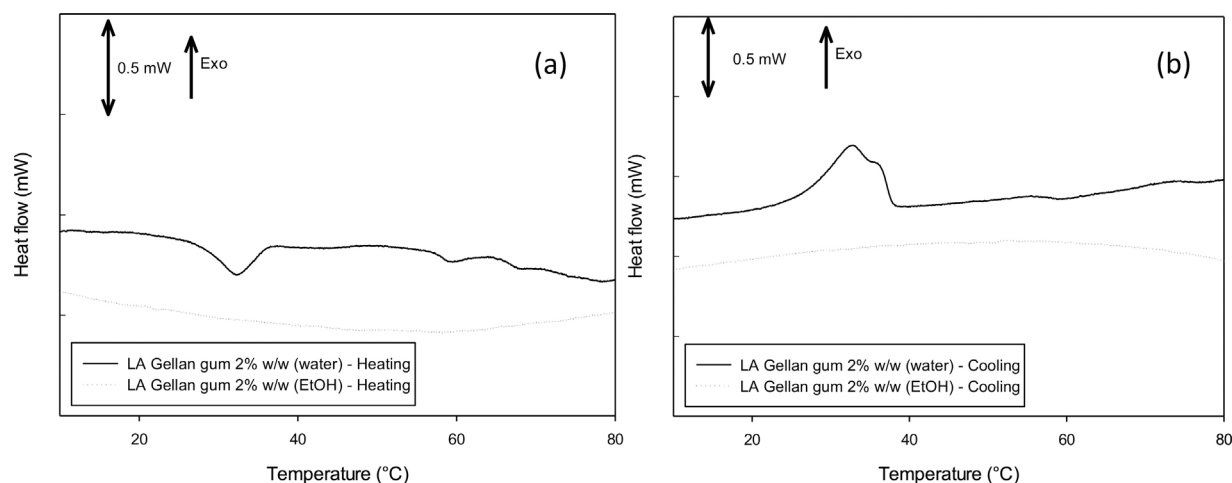


Fig. 4. a-b DSC curves: LA gellan gum on heating (a) and cooling (b).

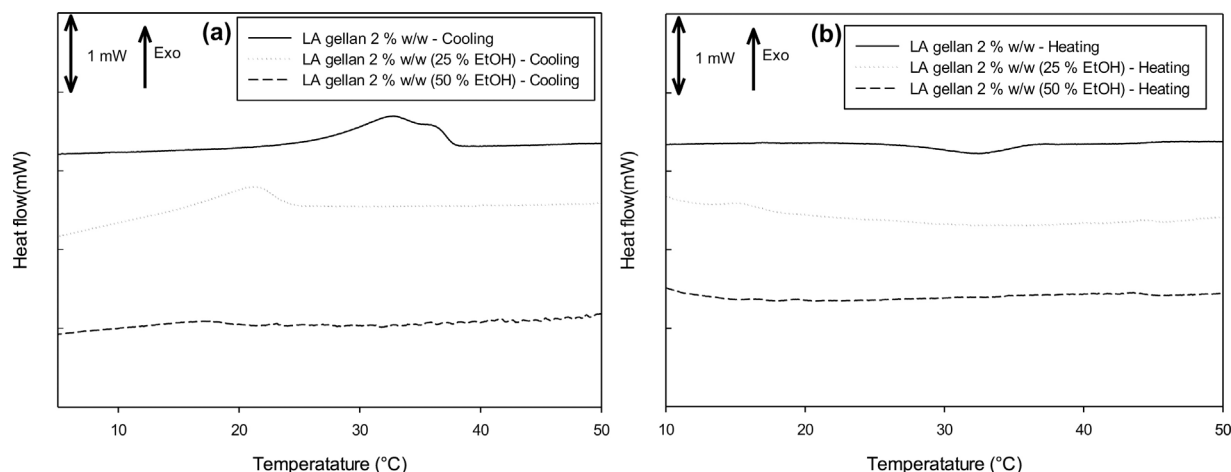


Fig. 5. a–b DSC curves: LA gellan gum treated with different ethanol concentrations on cooling (a) and heating (b).

In Fig. 5b the thermographs on heating are reported. The peak loss on heating at 25% was not expected, since a more disordered structure was anticipated, leading to a larger peak related to the non-aggregated domains. It suggested that the thermal transition temperature is not specific any longer, due to the alcohol addition.

A further increase in ethanol percentage led to a complete disappearance of the thermal transition, as concluded for the use of pure ethanol. The applied thermal cycles on mDSC analysis were within the temperature range of the gellan solid-state for both the untreated and treated samples.

The effect of the solvent molecule length and the OH position on the gel network were assessed. For this purpose, both isopropanol and 1-propanol were used.

In terms of mDSC analysis, these alcohols led to a flat thermal event within the same temperature range, following a trend similar to pure ethanol. Likewise, the collected infrared spectra show that the interaction between alcohols and the gellan gum network occurred. In effect, the typical gellan peak referred to the glycosidic bond at 1637 cm^{-1} shifts to 1611 cm^{-1} for 1-PrOH and to 1620 cm^{-1} for 2-PrOH (Fig. 6a–b).

Interestingly, peaks were noticed within the wave number range between 1100 cm^{-1} and 1000 cm^{-1} for 2-PrOH (Fig. 6b). Specifically, intense new peaks were found at 1069 cm^{-1} , 1055 cm^{-1} . Moreover, a shift from 950 cm^{-1} to 970 cm^{-1} was noticed. It may indicate that there is an effective interaction between LA gellan gum and isopropanol.

Therefore, in terms of the molecular level it seems that alcohols effectively interact with LA gellan gum.

3.2. Macroscopic level

Both the material texture and shrinkage evaluated in order to link them to the smaller-scale level. In particular, two different routes were undertaken, namely directly using a specific concentration of solvent or gradually adding it. In terms of shrinkage, it was measured that a gradual treatment was more suitable to better retain both the initial shape and volume, as it is shown in Fig. 7.

It was evident that a gradual treatment led to a shrinkage of $13.1\% \pm 0.2\%$, while the direct use of pure ethanol increased it up to $50.2\% \pm 0.6\%$. It is likely that the diffusion currents during the solvent exchange from outside inwards the material resulted in a gel network distortion (Bancroft & Gamble, 2008). Similar effects were noticed for isopropanol and 1-propanol.

Mechanical properties were clearly influenced by the solvent quality. Fig. 8 shows how the gel strength increases as the solvent concentration was raised. In addition the true fracture strain moved from $35.3\% \pm 1.7\%$ for non-treated gel to $42.7\% \pm 2.9\%$ for the alcogel treated with an ethanol gradient up to 100 wt%.

A microstructural explanation of the shrinkage and gel strength increase may be related to an increment in network packing and chain entanglement (Tkalec et al., 2015). However, it was found that the non-treated gels with the equivalent polymer density of the gradually treated alcogels (13.1% shrinkage) showed a strength only equal to $23.9 \pm 1.5\text{ N}$, well below the value referred to the alcogels (Fig. 8). The considerable strength increase might be due to the fact that alcohols act as fixatives, producing material hardening and shrinkage (Buesa, 2008; Titford & Horenstein, 2005).

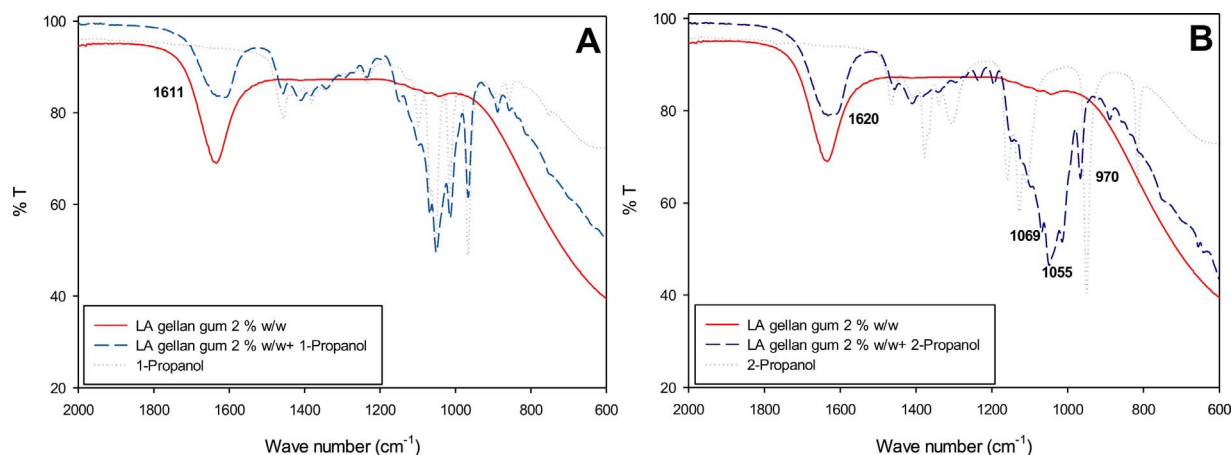


Fig. 6. a–b FT-IR spectra for LA Gellan gum with 1-propanol (a) and isopropanol (b). The peaks values are related to the alcogel.

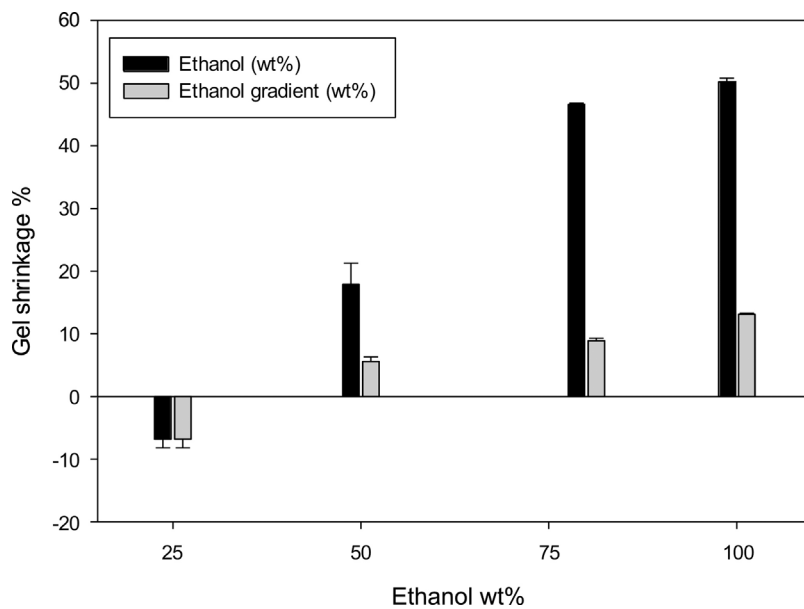


Fig. 7. Ethanol treatment: directly by using a specific concentration (wt%) (black bars) and through ethanol gradient (grey bars).

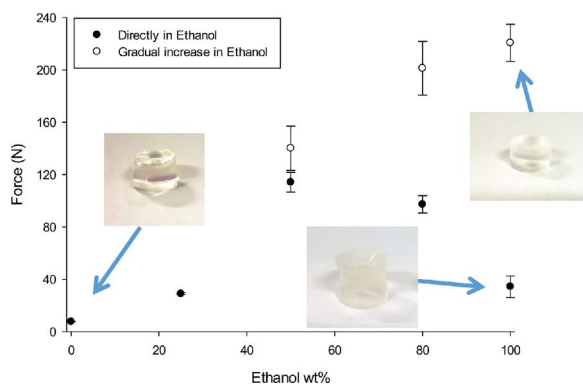


Fig. 8. Gel strength for LA Gellan gum treated with ethanol.

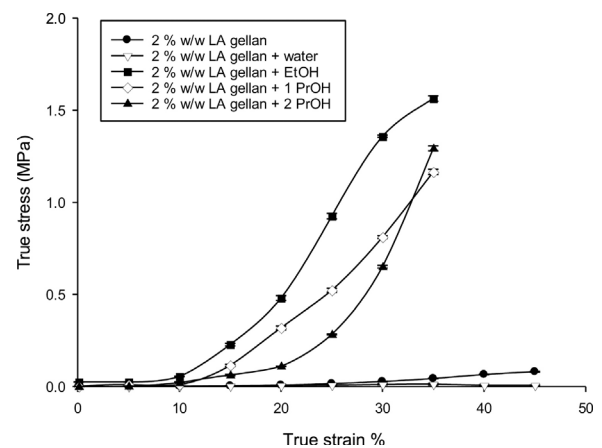


Fig. 10. True stress vs true strain for LA gellan gum as a function of solvent quality after complete gradual treatment.

On the other hand, this mechanical behaviour is likely not to be related to a more ordered structure, as previously discussed in the mDSC section. Specifically, these results may indicate that the slight alcogel turbidity noted in Fig. 8 might be dependent on a more packed network, rather than an increase in junction zones size (Banerjee & Bhattacharya, 2011), which means a more ordered system.

It is noteworthy to mention that the gel strength drop noted for direct use of solutions at ethanol concentrations greater than 50 wt%

was likely to be related to both the shrinkage and cylindrical shape distortion, when the gradual solvent treatment was not applied (Fig. 8). This was obviously an artefact for the measurement. This geometrical distortion was mainly due to the high solvent exchange through a stiffer outer portion of material caused by a high concentrated alcoholic

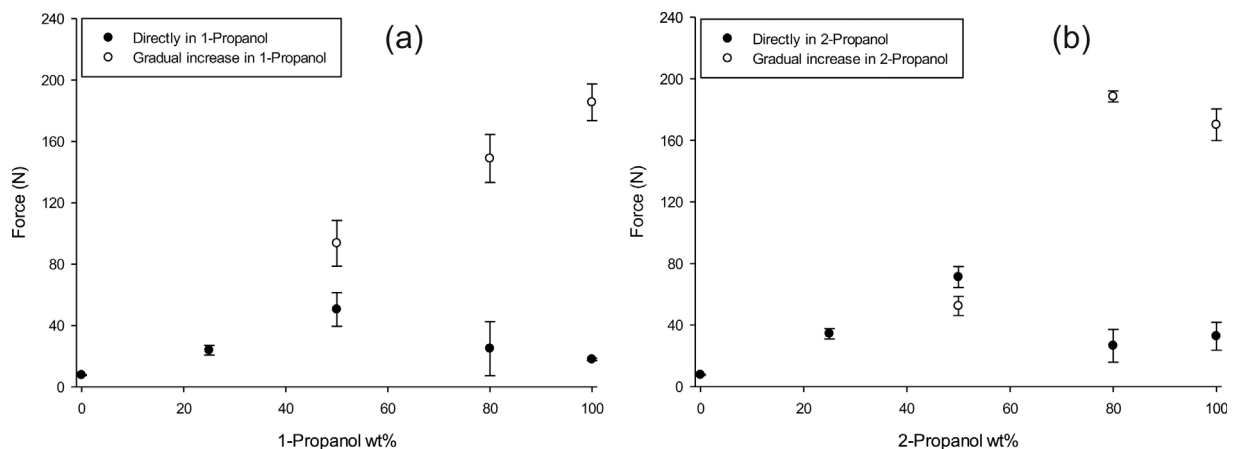


Fig. 9. a-b Gel strength for LA Gellan gum treated with 1-propanol and 2-propanol.

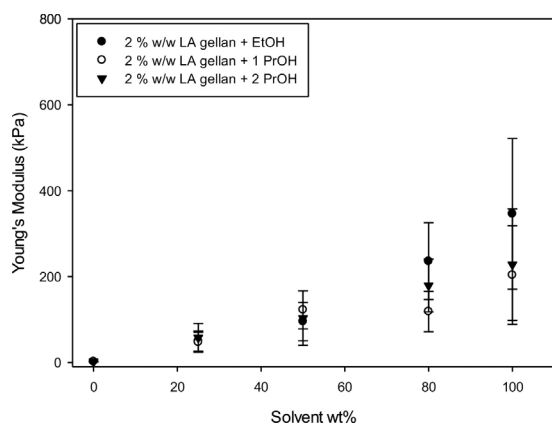


Fig. 11. Young's modulus for LA Gellan gum as a function of the solvent concentration. The collected data are related to a gradual solvent treatment up to the specific concentration.

solution that rapidly fixed the polymer chains (Buesa, 2008; Titford and Horenstein, 2005).

Increasing the solvent molecular length, the gel hardening was still evident, yet a slight decrease in the gel strength was noticed compared to ethanol, as it was possible to see when a gradient up to 100% isopropanol/1-propanol was used (Fig. 9). Similarly, the measured fracture true strain was $40.4\% \pm 2.8\%$ for the alcogel treated with a 1-propanol gradient up to 100 wt% and $39.5\% \pm 3.2\%$ for isopropanol.

Since the gel shrinkage for isopropanol ($13.9\% \pm 0.3\%$) and 1-propanol ($15.7\% \pm 2.5\%$) was comparable with gradual treatment

with ethanol ($13.1\% \pm 0.2\%$), the slight strength decrease might be dependent on a different polymer chain mobility and tribology as a function of the solvent molecule length, as Mills, et al. (2013) suggested. Moreover the different solvent viscosity (Spiro, Kandiah, & Price, 1990) might have a role in the gel mechanics. In agreement with the previous results, Fig. 10 compares the true stress and true strain as a function of the employed solvent after complete (up to 100 wt% alcohol) gradual solvent treatment. The mechanical properties results for the untreated LA gellan gum were in agreement with Norton, et al. (2011). It was noticed that at around 10% true strain the EtOH alcogel started to considerably increase the resistance to the compression, since there was an increment in true stress. On the other hand, this stress increase was slightly shifted to higher strain values for both 1-PrOH and 2-PrOH alcogels.

In Fig. 10 the curve related to gellan/water is also reported: the gel samples were submerged in pure water for 24 h before the texture analysis. A slight decrease in true stress was measured in comparison with the untreated gel. Combining this consideration to the slight volume expansion when more water was added during the treatment (25% ethanol + 75% water) (Fig. 7), it seemed that water tended to open the gel structure. More free water led to a gel softening, as shown in Fig. 10, in a sort of “network dilution”. It is noteworthy to mention that the error bars are collapsed to the experimental points due to the wide experimental range of true stress.

The increase in true stress as well as the Young's modulus (Fig. 11) with the solvent concentration suggested an entangled and packed structure when solvents were gradually used, raising the final stiffness value. Nevertheless, the network was less ordered, as discussed in the mDSC section.

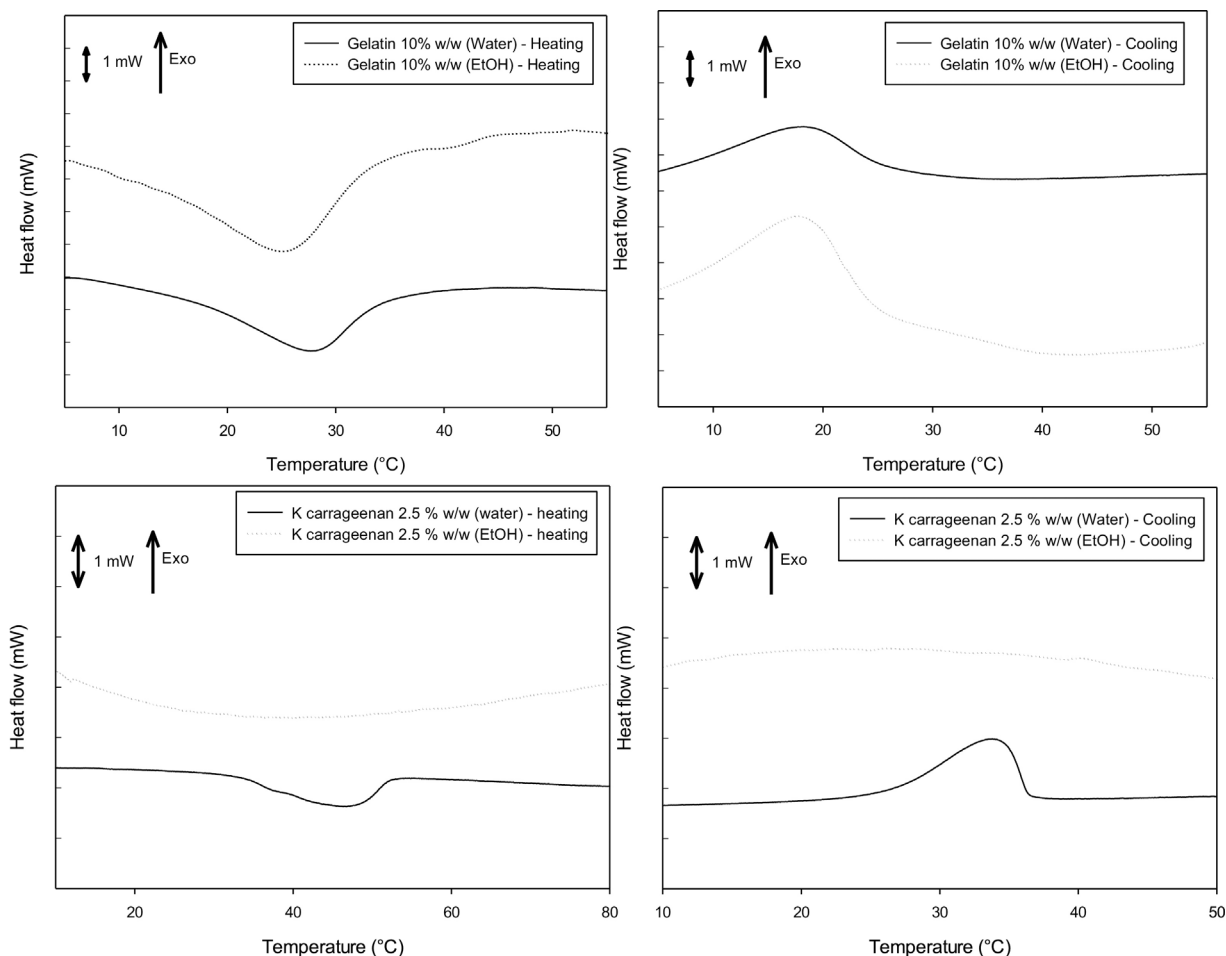


Fig. 12. DSC curves: gelatin (top) and k-carrageenan (bottom) on heating and cooling.

The error bars (plus/minus a single standard deviation) in Fig. 11 become more evident increasing the solvent concentration. Although the effect of the solvents on the quiescent gel shape distortion was considerably less pronounced if compared with the non-gradually-treated alcogel shape, it could affect the texture results.

In general, the gel strength increased as a function of the solvent concentration due to the molecular interactions between the alcohol and polymer, as the FTIR analysis suggested. It seems that alcohols do not affect the M^+ site available along the gellan gum chain (Fig. 1), since it is in contrast to the HA gellan gum mechanical properties. The acyl substituents (glycerate and acetate) (Fig. 1) are well-known to lead to a softer and more flexible gel (Phillips & Williams, 2000). Specifically, the glycerate provides stabilisation by adding new hydrogen bonds, yet disrupting of the binding site for cations by orientation change of the adjacent carboxyl group (Chandrasekaran & Thailambal, 1990; Morris et al., 2012) and consequently the junction zone alteration. On the other hand, the acetate hinders the helix aggregation (McClements, 2015; Morris et al., 2012). However, the acetyl groups do not modify the overall molecular network and the double helix structure (Chandrasekaran & Thailambal, 1990), unlike the alcohol case.

3.3. Comparison with gelatin and k-carrageenan

As an evidence and to further validate the considerations on the molecular level of LA gellan gum, a mDSC evaluation on k-carrageenan and gelatin was performed. These hydrocolloids were investigated as additional models, since they are respectively similar and different to LA gellan gum in terms of gelation and molecular configuration.

The collected mDSC results (Fig. 12) suggested that k-carrageenan had a similar behaviour to LA gellan gum, as it is shown in the thermographs (loss of defined thermal transitions), whereas gelatin preserves the thermal peaks. This trend was expected for k-carrageenan, considering that the polymer gelation is equivalent.

A further study might investigate the effect of ethanol on the gelatin-LA gellan gum gel mixture (Banerjee & Bhattacharya, 2011; Wang, Gong, Lin, Shen, & Wang, 2008) to assess which trend will be predominant.

4. Conclusion

The alcohol addition to gels was found to lead to water network alteration, which irreversibly affected the gel properties at both the molecular and macroscopic scales, depending on the solvent type and concentration. The reason for this behaviour is likely to be related to the interaction between the gel network and the solvent molecules.

From a macroscopic level, the presence of alcohols led to an increase in compression strength and stiffness due to the network fixation. Furthermore, depending on the alcoholic molecule length, the mechanical properties slightly changed, probably due to a different polymer chain mobility.

A gradual treatment allowed a more successful retention of both the volume and shape with respect to the direct use of solutions at high alcohol content. Therefore, it is recommended when gelling agents are combined with alcohols.

Finally, it seems that this study can be extended to other gelling agents and it is likely to expect similar results if the gels are comparable to gellan gum, like k-carrageenan, or different results if the polymer and its gelation are dissimilar.

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